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***Plasmodium berghei*-Hamster Cheek Pouch Model for the study of Severe Malaria**

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Running Head: Hamster cheek pouch malaria model

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) Severe malaria in man, caused by <i>Plasmodium falciparum</i> , is typically characterized by drowsiness, disorientation, incoherence, and severe headache. Untreated, coma and death often follow; mortality may be as high as 25% to 50%. The syndrome is often called cerebral malaria because of its most common clinical signs and typical postmortem cerebral lesions; however, it involves multiple organ systems, and results in far-ranging pathologic change. Systemic changes include an adult respiratory distress-like syndrome, acute renal tubular necrosis, hypoglycemia, thrombocytopenia, dyserythropoiesis and hemolysis, gastrointestinal abnormalities, and liver damage. Simple invasion of erythrocytes (RBCs) by protozoa cannot explain all of the lesions described. Several hypotheses have been advanced to try to explain the observed lesions--increased permeability of the blood brain barrier, disseminated intravascular coagulation, immunologically mediated disease, and systemic toxemia--yet, the pathogenesis of severe malaria remains unknown. Yoeli, noting that the human vascular lesion can only be observed on postmortem, challenged			
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Severe malaria in man, caused by *Plasmodium falciparum*, is typically characterized by drowsiness, disorientation, incoherence, and severe headache. Untreated, coma and death often follow; mortality may be as high as 25% to 50%. The syndrome is often called cerebral malaria because of its most common clinical signs and typical postmortem cerebral lesions; however, it involves multiple organ systems, and results in far-ranging pathologic change. Systemic changes include an adult respiratory distress-like syndrome, acute renal tubular necrosis, hypoglycemia, thrombocytopenia, dyserythropoiesis and hemolysis, gastro-intestinal abnormalities, and liver damage. Simple invasion of erythrocytes (RBCs) by protozoa cannot explain all of the lesions described. Several hypotheses have been advanced to try to explain the observed lesions — increased permeability of the blood brain barrier, disseminated intravascular coagulation, immunologically mediated disease, and systemic toxemia — yet, the pathogenesis of severe malaria remains unknown. Yoeli, noting that the human vascular lesion can only be observed on postmortem, challenged malariologists to develop an *in-vivo* model in which one might observe and record the changes in the microcirculation during active infection.

## ANIMAL MODEL

The classical hamster cheek-pouch model<sup>12,13</sup> has now been combined with a previously described *Plasmodium berghei*-infected golden hamster model. The vascular lesions observed in the cheek pouch of the living anesthetized hamster (Figures 1-3) and in the post-mortem cheek pouch (Figure 4) are similar, in degree of severity and time of onset, to those occurring in the hamster brain.<sup>15</sup> Golden hamsters (*Mesocricetus auratus*) of either sex are infected at 4-6 weeks of age with  $1.5 \times 10^7$  *P. berghei*-parasitized (ANKA strain) hamster red blood cells injected intraperitoneally. Infected animals typically live for 12-15 days, during which time one can observe, *in vivo*, mononuclear cells containing



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intracytoplasmic malarial pigment, margined with adherent RBCs, in submucosal blood vessels; malarial parasitized RBCs; and progressive failure of capillary beds. Using the cheek pouch model, one can observe and manipulate the dynamic relationships between formed blood elements and the endothelium in severe malaria.

### COMPARISON WITH HUMAN DISEASE

Postmortem brain lesions in man include petechia in the white matter; congestion of capillaries and venules; aggregates of parasitized RBCs, macrophages, and mononuclear leucocytes containing malarial pigment and interwoven by fibrin; perivascular hemorrhages; glial granulomata; ischemic changes in nerve cells; and sometimes edema.<sup>16</sup> All these lesions (with the exception of glial granulomata) can be observed in the hamster brain post-mortem, only the relative frequency of occurrence being different. Congestion of RBCs in cerebral capillaries, normally noted as the most striking postmortem histologic sequela of severe malaria in humans, was infrequently seen in our studies or those described by Rest and Wright.<sup>17</sup> Mononuclear cells containing malarial pigment are commonly seen along the walls of venules within the cheek pouch and brain; these cells are occasionally observed in the brains of cerebral malaria patients<sup>16</sup> and alveolar capillaries of *P.-falciparum* malaria patients who die with acute pulmonary insufficiency.<sup>18</sup> Like its human counterpart, the *P. berghei* infection in hamsters is a lethal systemic disease involving vascular beds other than those of the brain; we have also demonstrated mononuclear/RBC aggregates in the vascular beds of the lung, liver, spleen, and kidney in hamsters.<sup>15</sup>

## USEFULNESS OF THE MODEL

This model allows simultaneous observation of morphology and function in a large vascular bed; pathologic change may be recorded by photo- or videomicroscopy. Lesions in small capillaries are generally observed in the pouch *in vivo* before they can be seen by histologic techniques. The broad perspective gained facilitates focus of effort for subsequent histopathology or pharmacology. Compounds that might alter the vascular lesions can now be screened quickly while observing their *in-vivo* effects. Individual cells or vascular regions may be collected for further study. This model is ideally suited to study the role of the monocyte in severe malaria, as this cell type is sequestered in greater numbers in hamster vessels than in the vessels of the brains of humans. There has recently evolved a general acceptance of the importance of monokines in *Plasmodium* infection. Antibody directed against recombinant tumor necrosis factor (TNF)<sup>19</sup> or pretreatment with dexamethasone<sup>20</sup> can prevent the development of the described lesions in rodents, while recombinant TNF given to mice with low *P. vinckei* parasitemia leads to clinical signs like those seen in high parasitemia.<sup>21</sup> Lastly, the finding of elevated serum levels of TNF in 7 of 10 malarial patients<sup>22</sup> and the growing body of evidence suggesting a significant role for cell-mediated immunity in the pathogenesis of human malaria<sup>23</sup> make this model of severe human malaria even more attractive and useful.

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## LEGENDS FOR FIGURES

Figure 1. Mononuclear cell (at arrow), containing malarial pigment, adherent to the endothelium of a 30- $\mu$ m vessel within the cheek pouch of a living hamster infected with *P. berghei*. When noted, such cells remain attached in one position and blood flow continues around them throughout the entire period of observation, typically 15-60 min. Several RBCs appear to be adhering to the larger mononuclear cell. In the background are skeletal muscle fibers, normally seen in the pouch preparation.

Figure 2. A venule of 40- $\mu$ m diameter within the cheek pouch of a living hamster 6 days after challenge with *P. berghei*. RBCs are commonly seen adhered to the walls of venules and small veins, although the walls of capillaries and arterial vessels are universally free of cells. Such RBCs are sometimes seen in the vessels of noninfected animals, but the venular and venous walls of infected animals' pouches are typically densely covered with cells, especially between 5-7 days post-challenge, resulting in a cobblestone appearance. Some of the spherical cells tumble slowly along the vessel walls, propelled by flowing blood. Tumbling cells move at different rates as if some adhere more tenaciously than others.

Figure 3. Venous (v) and arterial (a) vessels within the pouch of a living hamster 12 days after *P. berghei* challenge. Note the typical aggregate of cells, probably pigment-laden mononuclear cell(s) with numerous RBCs adherent (at bracket), apparently attached to venous endothelium. Formed elements can be seen as blurred eddy currents passing around the lesion. One can observe *in vivo* as additional RBCs leave the flowing blood to adhere momentarily or permanently to the surface of the "growing" lesion.

Figure 4. Photomicrograph of azure II methylene blue 1- $\mu$ m section from the cheek pouch of a *P. berghei*-infected hamster killed 12 days post-challenge. Note endothelial walls (e)

of a vessel containing parasitized (p) and nonparasitized (np) RBCs with marginated mononuclear cells (m), possible mediators of endothelial cell damage, near disrupted endothelium on the lower margin. RBCs, primarily nonparasitized, can be seen outside the vessel representing hemorrhage. The lesion is similar to the "ring hemorrhages" described in the brains of humans after death due to *P. falciparum* infection. Such hemorrhages are typically not seen in the cheek pouch *in-vivo* preparation. x630.

NOTE: Figures used with permission from Franz, et al., 1987 <sup>15</sup>

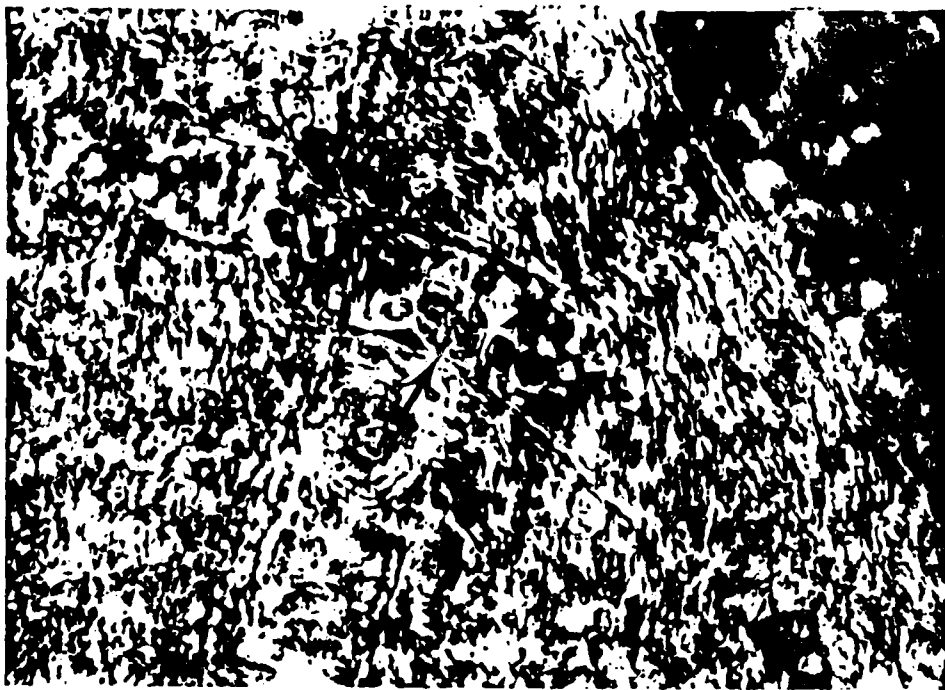


Fig 1

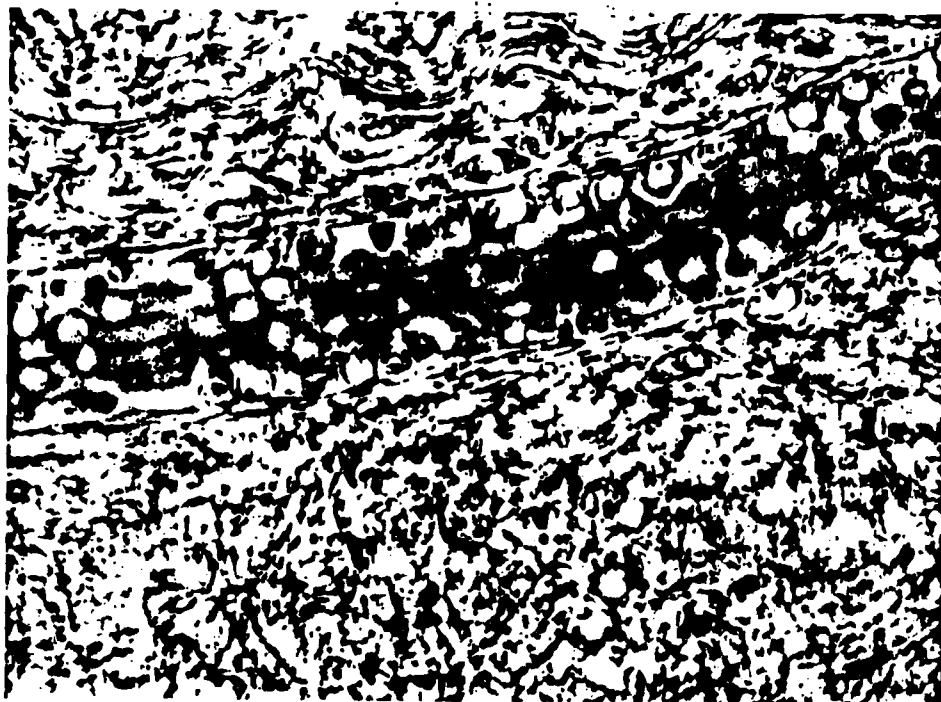


Fig 2



Fig 3

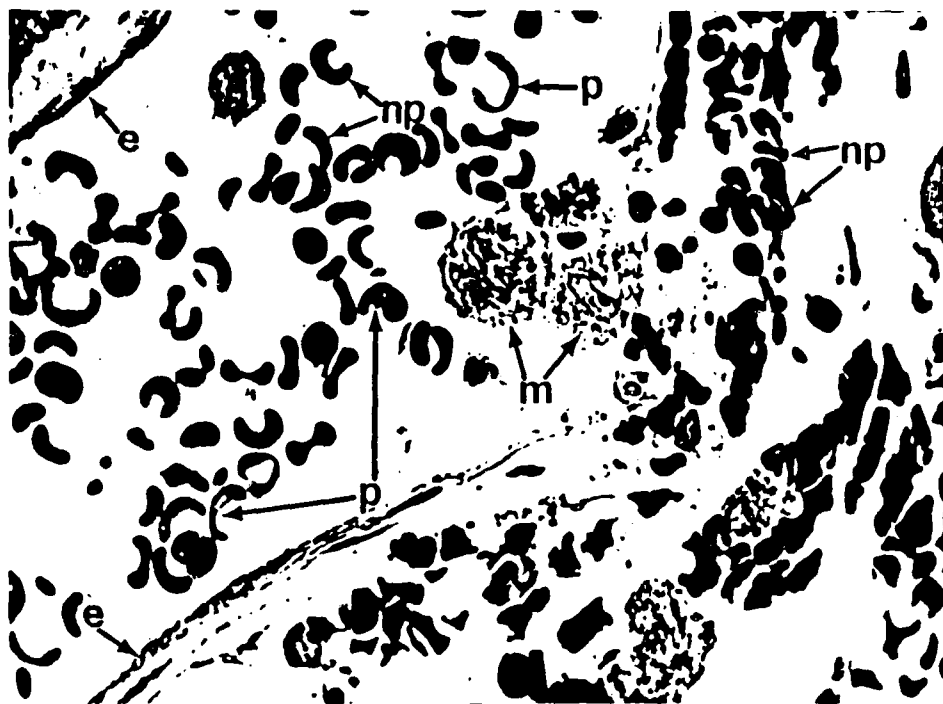


Fig 4